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Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass

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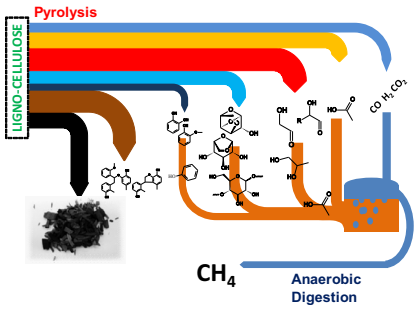
HIGHLIGHTS

- Aqueous fraction from pyrolysis inhibits anaerobic digestion.
- Inhibition is revealed by a volatile fatty acids accumulation and long lag phase.
- Addition of biochar in the system decreases inhibition and doubles yield.
- Semi-continuous system pre-loaded with biochar shows acceptable performances.

GRAPHICAL ABSTRACT



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ABSTRACT

Intermediate pyrolysis produces a two-phase liquid whose aqueous phase is characterized by low heating value and high water content (aqueous pyrolysis liquid, APL). Anaerobic digestion can be the straightest way to produce a fuel (methane) from this material. Batch tests showed poor performance in anaerobic digestion of APL, which underlined the inhibition of biological process. Nutrient supplementation was ineffective, whereas biochar addition increased yield of methane (60 ± 15% of theoretical) with respect to pure APL (34 ± 6% of theoretical) and improved the reaction rate. On the basis of batch results, a semi-continuous biomethanation test was set up, by adding an increasingly amount of APL in a 30 ml reactor preloaded with biochar (0.8 g ml^{-1}). With a daily input of 5 g d^{-1} l^{-1} of APL (corresponding to overall amount of 0.1 kg l^{-1} added before the end of the study) the yield of methane was 65 ± 5% of the theoretical.

1. Introduction

Pyrolysis can be considered one of the fastest way for depolymerization of biomass macromolecules to smaller molecular fragments. Intermediate pyrolysis, or pyrolysis with temperature of 400–500 °C and reaction time of about 10–30 s, thanks to low heat transfer required, can be readily performed with simple reactor configurations. For this reason it can be considered economical also at small scale, and it is able to yield char (20–30%), gas (10–20%) and 50–60% w/w of a pyrolysis liquid with relatively high (about 50% w/w) water content (Bridgwater, 2012).

In intermediate pyrolysis, due to the high amount of reaction water produced, phase separation usually occurs. This phenomena ends up with a water insoluble "tarry like" fraction (bottom phase consisting of 15–30% w/w_{liquid}) and an aqueous syrup (85–70%, hereinafter called aqueous pyrolysis liquid, APL) formed by water, water soluble substances and slightly soluble substances (e.g. phenols and furans) characterized by low calorific value and not ignitable (Oasmaa and Kuoppala, 2008). Even if diluted and with low heating value, this fraction can consist in more than 70% of the pyrolysis liquid weight and about 30% of its calorific value. Being

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already phase separated, most of the APL is water soluble, and it is formed by C2–C6 sugars, hydroxyacids, oligomers and water soluble phenols (e.g. catechol), which could be envisaged as a feedstock for biological processing (Cordella et al., 2012).

The intrinsic advantage of biological processing is that bacteria are able to convert, at low temperature, a large array of compounds with different chemical features to few or single product which can be obtained in high purity form. For instance, fermentative pathways are able to de-oxygenate completely sugars molecules with extraordinary high selectivity, if compared to chemical pathways (Brown, 2007). On the other side, the major challenge is that APL contains several compounds, at percent level, which are toxic to microorganisms. Then the process should be optimized to exploit biological transformation, overcoming the toxic effect of pyrolysis derived chemicals. This idea translates into the so-called "hybrid thermochemical-biological concept" which was recently investigated for production of ethanol from second generation biomass (Jarboe et al., 2011).

One of the simplest available biological conversion is anaerobic digestion, that consists in a series of interconnected biological conversions that end up with the production of a gaseous mixture of carbon dioxide and methane, which can be purified and used as drop-in fuel (Scholz et al., 2013). This conversion can be performed into low cost and non-sterile equipments by-means of mixed anaerobic consortia which can be, in principle, adaptable to the widest range of chemical substrates (Appelsa et al., 2011). Moreover, being the product gaseous, anaerobic digestion allows to operate at higher level of dilution in comparison to other fermentation processes (e.g. ethanol production) that requires extraction of the product from the broth. According to these considerations, anaerobic digestion can be considered one of the simplest way to produce a drop-in fuel through APL.

In the past, pioneering work by Andreoni et al. (1990) studied the anaerobic digestion of pyroligneous acid (similar to APL) mixed with swine manure (for pH correction), showing that the conversion is feasible, even at relatively high concentrations (up to 100 g l^{-1}) upon some process modifications (e.g. use of wood-chips as filling material). More recently, Willner et al. (2004) studied the digestion of pure pyrolysis-oil and demonstrated the feasibility of digestion of 1 g l^{-1} pyrolysis-oil, but found a complete intoxication in presence of 20 g l^{-1} pyrolysis-oil. Besides, the mechanisms of acclimatization and recovery of methanogenic activity in continuously operated anaerobic systems are poorly understood.

The low number of papers on a potentially interesting topic could be easily explained considering that the progress in commercialization of anaerobic digestion plants and in pyrolysis/gasification technologies is something relatively recent, as well with the fact that, in academia, thermochemical approaches are often parallel or competing with biochemical ones. Nevertheless, it is now indubitable that the demonstration, under certain conditions, of pyrolysis-oil biomethanation, considered with the wide evidence of syngas and producer gas biomethanation (Guiot et al., 2011), would open new opportunities for process integration. From one side, pyrolysis could help anaerobic digestion, disclosing refractory material to biological conversion and separating organic from inorganic fraction. From the other side, anaerobic digestion can be envisaged as a way to exploit the low calorific value fractions (e.g. APL) of pyrolysis liquids or, in the gasification process, for conditioning and clean up of raw producer gas (syngas and condensable material). Therefore, the aim of this work is to evaluate in detail the anaerobic digestion of APL and to indentify the actual inhibition mechanisms. In particular, the addition of biochar (co-produced by means of 400 °C pyrolysis) was studied as a simple solution for increasing of the performance of the system.

2. Methods

2.1. Pyrolysis of biomass

Corn stalk pellets were pyrolyzed at 400 °C for 10 min in a fixed bed reactor, described in detail elsewhere (Fabbri et al., 2007). The sample, heated up to the furnace temperature at a heating rate of about 100 °C min^{-1}, was maintained at 400 °C for 10 min. Pyrolysis was performed on 5 g samples, and the entire procedure was repeated 5 times in order to collect the amount of oil needed for anaerobic digestion tests and chemical characterization. The amount of char produced was measured as the weight of the solid material still present at the end of the pyrolysis run. The amount of pyrolysis liquid produced was calculated as the difference between the weight of the trap system before and after the pyrolysis run. The liquid product was an heterogeneous mixture of aqueous and tarry fractions. In order to have homogenous aqueous sample, the mixture was centrifuged for 30 min at 300 *g* and the APL was recovered as a dark red syrup. APL was stored at −20 °C and used without any further treatment. The pyrolysis of 25 g of biomass yielded 11.25 g of solid residue (biochar) and 12.3 g of liquid product consisting in 3.0 g of tarry like insoluble product and 9.3 g APL. Biochar and aqueous products, obtained from the same pyrolysis, was used for all the anaerobic digestion tests. Chemical characterization was performed as described in Cordella et al. (2012) and Busetto et al. (2011). The theoretical oxygen demand (ThOD, gThOD g^{-1}), accordingly to OECD guideline 301F, was calculated from elemental analysis using the following formula:

$$\mathrm{ThOD} = 16 \cdot ((2 \cdot \mathrm{C}/12 + \mathrm{H}/2 + 2 \cdot \mathrm{S}/32 - \mathrm{O}/16 - 3 \cdot \mathrm{N}/28)$$

where C, H, S, N and O are respectively the % w/w of carbon, hydrogen, sulfur, nitrogen and oxygen.

The biological oxygen demand (BOD, gBOD g^{-1}) of the APL was determined by a ready biodegradability test in an aerobic aqueous medium according to the OECD guideline 301F, "Manometric respirometry" for 28 days at 20 ± 2 °C, using bacterial inoculum from an activated sludge taken from a treatment plant receiving domestic sewage located in Ravenna (Italy). Main chemical characteristic of the APL were summarized in Table 1.

2.2. Fed-batch anaerobic digestion of APL with un-adapted inocula

The first phase of inoculum adaptation was performed on 1 l reactors at 40 °C, which was inoculated with 950 ml inoculums taken from various sources (namely corn silage digestion plant, distillery residues digestion plant and MSW digestion plant) in order to have the maximum potential for APL conversion The inoculum total suspended solid (TSS) was 51 g l^{-1}, the volatile suspended solids (VSS) was 41 g l^{-1} with a COD of 47 gCOD l^{-1}. The test was conducted by three consecutive spikes of substrate at time zero (15 g of oil) and every 60 days (22 g each spikes), corresponding of an organic load of 10 and 15 gCOD l^{-1} of reactor and providing a food to microorganism ratio (F/M, $gCOD_{substrate}$ $gCOD_{VSS}^{-1}$) equal to 0.2 and 0.3 .

Reactor consisted in stainless steel lightly pressurized reactor and was loaded with three consecutive input of APL. Gas amount and composition was analyzed continuously by automatic datalogger and approximate pH was checked, after APL addition, by means of litmus test.

2.3. Detailed study of the APL digestion process in micro-batch tests

For a detailed study of anaerobic digestion of APL, 100 ml syringe reactors were used, according to the Hohenheimer Biogas Yield Test (Mumme et al., 2014). This allowed to test large number

Table 1
Chemical characterization of the APL used for anaerobic digestion tests.

	Aqueous pyrolysis product
Yield% w/$w_{corn\ stalk}$	37
C% w/w	19
H% w/w	11
N% w/w	0.3
S% w/w	–
O% w/w	70
Ash% w/w	0.1
C/N molar ratio	73
ThOD (gCOD g^{-1})	0.70
BOD (gBOD g^{-1})	0.62
Theoretical MP (L CH_4/g)[a]	0.21
	% g g^{-1}
Water	55
WS-EI[b]	30
WS-ES[c]	15
PL[d]	0.5
HE[e]	0.2
	g kg^{-1}
Acetic acid	26
Propionic acid	1.6
Isobutyric acid	0.1
Butyric acid	0.5
Isovaleric acid	3.2
Valeric acid	0.1
Hydroxyacetaldehyde	19
Small oxygenated compounds	42
Hydroxyacids	6.0
Anhydrosugars	4.2
Phenols	17
Furans	14
N-compond[f]	2.4

[a] Theoretical methane potential (MP) calculated from the elemental analysis.
[b] Water soluble/ethylacetate insoluble from solvent fractionation scheme.
[c] Water soluble/ethylacetate soluble from solvent fractionation scheme.
[d] Pyrolytic lignin from solvent fractionation scheme.
[e] Hexane soluble fraction.
[f] Nitrogen containing compounds.

of concentration for APL in a preliminary study and, after that, to follows volatile fatty acids (VFA) concentration and methane production from batch test performed with biochar and nutrients.

Five duplicate samples were prepared for, namely:

1. 30 ml inoculum (from 1 l fed-batch digestion of APL) and 1.5 g of APL corresponding to a concentration of 5% w/w, 35 gCOD l^{-1}, 0.6 F/M ratio.
2. 30 ml inoculum (from 1 l fed-batch digestion of APL), 60 mg of ammonium chloride (NH_4Cl), 25 mg of potassium phosphate (K_3PO_4) and 1.5 g of APL, corresponding to a concentration of 5% w/w, 35 gCOD l^{-1}, 0.6 F/M ratio.
3. 30 ml inoculum (from 1 l fed-batch digestion of APL), 1.5 g of biochar and 1.5 g of APL, corresponding to a concentration of 5% w/w, 35 gCOD l^{-1}, 0.6 F/M ratio.
4. 30 ml inoculum (fresh inoculum) and 0.3 g of glucose. corresponding to12 gCOD l^{-1} and 0.3 F/M, equal to 0.3. Positive control.
5. 30 ml inoculum (from 1 l fed-batch digestion of APL)

Inoculum for APL digestion was taken from 1 l fed-batch tests with the following characteristics: TSS was 55 g l^{-1}, the VSS was 45 g l^{-1} with a COD of 53 gCOD l^{-1}. For glucose digestion fresh inoculum (see Section 2.2) was used.

The reactor consisted in 100 ml plastic syringe (Fig. 1), a detailed visual description of the equipment and procedure can be found in Supporting information.

Briefly, the syringe plunger was removed and the syringe opening was closed with an airtight rubber cap. The closed half syringe was partially filled with a portion of the inoculum. At this stage, two syringes were loaded with nutrients and two syringes were loaded with grinded biochar obtained from pyrolysis of corn stalk. After, the syringe were reversed, the air was removed from inside by pushing the plunger until complete removal of empty space. After that, the syringe was closed with a rubber cap and kept at 40 °C for 10 days, as pre-incubation/ degassing. After pre-incubation, each syringe were finally loaded with feedstock and the pneumatic seal was checked by lightly pulling the plunger. Finally the syringe was placed in a thermostat room at 40 °C for all the duration of the study.

During the test, the produced biogas was collected in the expansion volume of the syringe, therefore the amount of biogas produced was evaluated by visual quantification of the volume produced (every 3–4 days). The composition of the gas was obtained by withdrawing 10 ml of gas through the rubber cap (by means of a needle) and analyzing it by means of GC-TCD (Agilent 78120A calibration with a). After each gas quantification and analysis, the rubber cap was removed, residual bio-gas and incoming air was expelled from the syringe and about 100 mg liquid sample (for VFA analysis) was obtained. After that, the syringe was closed, pneumatic seal was checked by lightly pulling the plunger, and the syringe was placed back in the thermostat room at 40 °C. At the end of the study, in order to consider the amount of substrate/inoculum removed for the VFA analysis (about 0.5 g per week, see below for details) the cumulate methane amount for each day were corrected by division of the methane amount by 1-A, whereas A is the cumulate sum of all the aliquot of

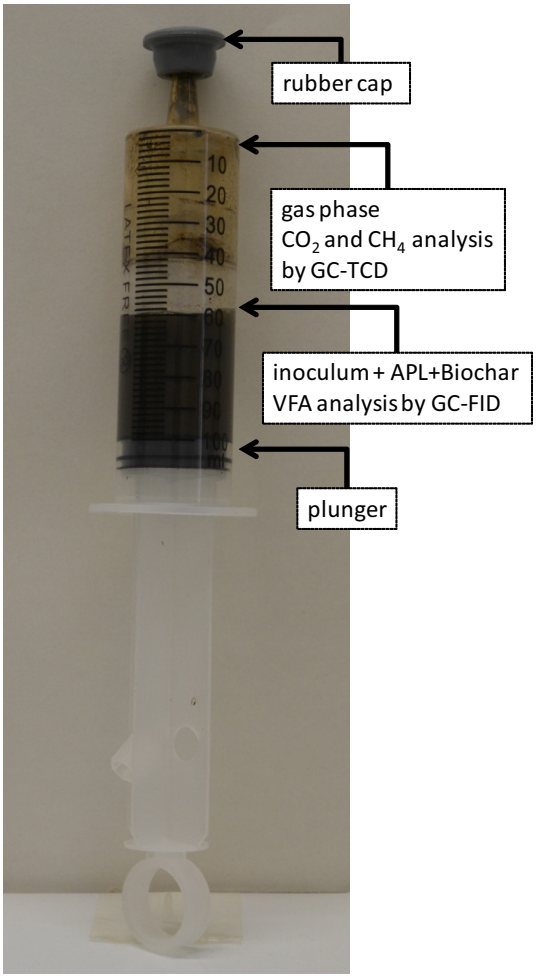

Fig. 1. Picture of the syringe reactor used for micro-batch tests.

inoculums removed from the system through the study. The chemical oxygen demand (COD) transformed into methane (COD_{CH_4}, gCOD l^{-1}) was calculated from density of gaseous methane (0.62 g l^{-1} at 40 °C) and its ThOD (4.0 gCOD g^{-1}) using the following formula:

$$COD_{CH_4} = 2.48 \cdot \frac{V_{CH_4}}{V_{reactor}}$$

where V_{CH_4} is the cumulative production of methane (ml) during the experiment and $V_{reactor}$ is the volume of reactor (e.g. 30 ml in the small batch test).

2.4. Semi-continuous tests of APL digestion

For semi-continuous test, four syringes were prepared following the procedure used for micro-batch tests (paragraph 2.3), but with semi-continuous addition of substrate. At the beginning of the study, the syringes were filled with 30 ml of the same inoculum used for micro-batch (previous paragraph) and 24 g of biochar obtained from pyrolysis of corn stalk were added to two syringes. The system was initially fed with small amounts of APL and subsequently stressed with increasingly high loading of APL.

During the test, the produced bio-gas was collected in the expansion volume of the syringe, therefore the amount of biogas produced was evaluated by visual quantification of the volume produced (every 3–4 days). The composition of the gas was obtained by withdrawing 10 ml of gas through the rubber cap (by means of a needle) and analyzing it by means of GC-TCD (Agilent 78120A). After each gas quantification and analysis, the rubber cap was removed, residual bio-gas and incoming air was expelled from the syringe and the syringe was loaded with an additional amount of APL, introduced by means of a micropipette through the syringe cone. After that, the syringe was closed, pneumatic seal was checked by lightly pulling the plunger, and the syringe was placed back in the thermostat room at 40 °C. All calculations were performed as in previous sections.

2.5. VFA analysis

Analysis of VFA was performed with single drop extraction procedure (SDE, Xu et al., 2007) optimized for analysis of VFA. The analysis aliquot (≈100 mg) was added with 0.1 ml of internal standard solution(2-ethylbutyrate in distilled water) and 0.2 ml of saturated $KHSO_4$ solution. Then 1.2 μl drop of dimethyl-carbonate, by means of a 10 μl chromatography microsyringe, was exposed to the solution for 20 min for SDE. After the SDE, the drop was retracted and injected in a GC-FID (injection temperature 250 °C) equipped with polar GC column (Agilent Q7221J&W nitroterephthalic-acid-modified polyethylene glycol DB-FFAP 222 30 m, 0.25 mm, 0.2 μm) with the following thermal program: 80 °C for 5 min, then 10 °C/min to 250 °C. Calibration was performed by applying the same procedure to standard solutions containing known amount of the five VFA analyzed (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid). The VFA concentration was expressed as chemical oxygen demand ($COD_{VFA,}$ gCOD l^{-1}) of VFA which was calculated from the ThOD of each VFA using the following formula:

$$COD_{VFA} = \sum_i ThOD_i \cdot VFA_i$$

where $ThOD_i$ (gThOD $g^{-1}_{substance}$) is 1.07, 1.51, 1.82, 2.04 for acetic acid, propionic acid, butyric/isobutyric acid, valeric/isovaleric acid respectively and VFA_i is the concentration of different VFA in g l^{-1}. All reagent and chemicals were purchased from Sigma–Aldrich and used without purification.

3. Results and discussion

3.1. Fed-batch anaerobic digestion of APL with un-adapted inocula

The first phase of inoculum adaptation was performed by means of 1 l fed-batch reactor, by means of three consecutives spikes of substrates.

Fig. 2 shows the methane production (mean of duplicate) during the 1 liter batch test. In the first phase, the reactor produced less biogas than control (without APL), which indicates a significant inhibition due to APL addition. Methane production was negligible for 20 days, after that, methane production started and surpassed the blank reactor with an average production of 100 Nml of CH_4 d^{-1} followed by a slowdown of gas production, with a final yield of less than 20 ± 2% (mean ± standard deviation, $n = 2$) of the theoretical biomethanation potential of APL added. After the second APL spike, a longer lag phase (50 d) and a sustained gas production (equal to 48 ± 5% of theoretical methane potential) was observed. After the third APL spike, the methane production stopped again for 70 days and the final gas production was almost negligible, indicating a large degree of inhibition and/or a ineffectiveness of the conversion.

Looking at the gas composition (Fig. 2), after each spike, even if the alkalinity of the inoculum was enough to buffer the acidity of APL (pH stood above 7 during the experiment) the production of methane stopped suddenly, and a large carbon dioxide production, probably due to shift in carbonates equilibrium, were observed. From overall methane production of the three consecutive batches performed, the final yield was about 27 ± 5% of the theoretical methane potential of APL added, which is largely below the amount of biodegradable substances content of APL (see Table 1, BOD/ThOD = 0.89). Since a large portion of the APL is not stable at the anaerobic digestion environment (e.g. pH > 7 and presence of alkalinity), the occurring of so long lag phases, and then toxicity/adaptation problems, can be, as well, a reason for poor yields obtained in these tests (Fahmi et al., 2008) .

Looking at the literature on inhibition of anaerobic processes, and according to the APL composition, the causes of the process slowdown can be due to inhibition from the APL constituents (which are well known inhibitors of fermentation), inhibition from APL fermentation products (e.g. VFA) or the lack in nutrients, especially phosphorous which is almost absent in the APL (ash content is negligible). In order to understand the cause of inhibition and possibly remove it, the study was deepened with additional small scale batch test, which were undertaken in the next paragraph.

3.2. Detailed study of the APL digestion process in micro-batch tests

A micro-scale test was set up in order to follow the acidogenesis and bio-methanation process, and to detect the unbalances between various processes. Three strategies of APL digestion were compared, namely: (i) biochar addition in (biochar:substrate ratio of 1:1), (ii) supplementation with nutrients, in order to have a C:N:P ratio of 100:5:1; (iii) addition of only APL in the system. The latter test can be considered a smaller replicate of the previously performed test on 1 liter reactors, with the additional information on the VFA. Fig. 3 shows the trend in the conversion of the different substrates into VFA intermediates and methane. All data reported are the mean value of duplicate, which showed an acceptable relative standard deviation roughly equal to 25%, and were explicited as gCOD l^{-1} in order to compare directly the various products in term of chemical energy.

For glucose, which is a non-toxic and easily degradable substrate, the results obtained with the syringe reactor were comparable with the literature (Vavilin et al., 1996), with moderate

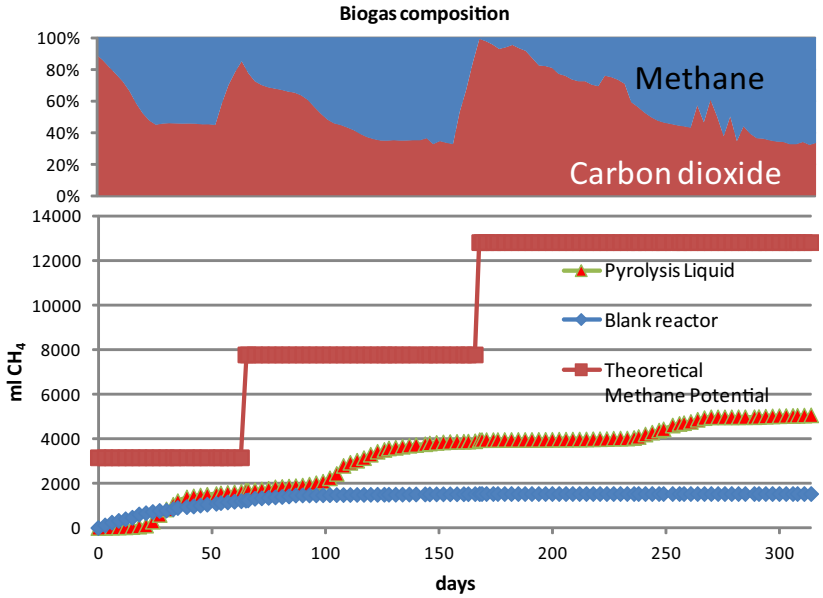

Fig. 2. Results from aqueous pyrolysis liquid (APL) digestion test, triangle (Δ) and rhombus (◇) shows the actual production of methane respectively from pyrolysis liquid added reactor and from blank reactor (only inoculum). Red squares (□) shows the amount of pyrolysis oil added expressed as theoretical methane potential (0.21 l CH_4/g pyrolysis liquid). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

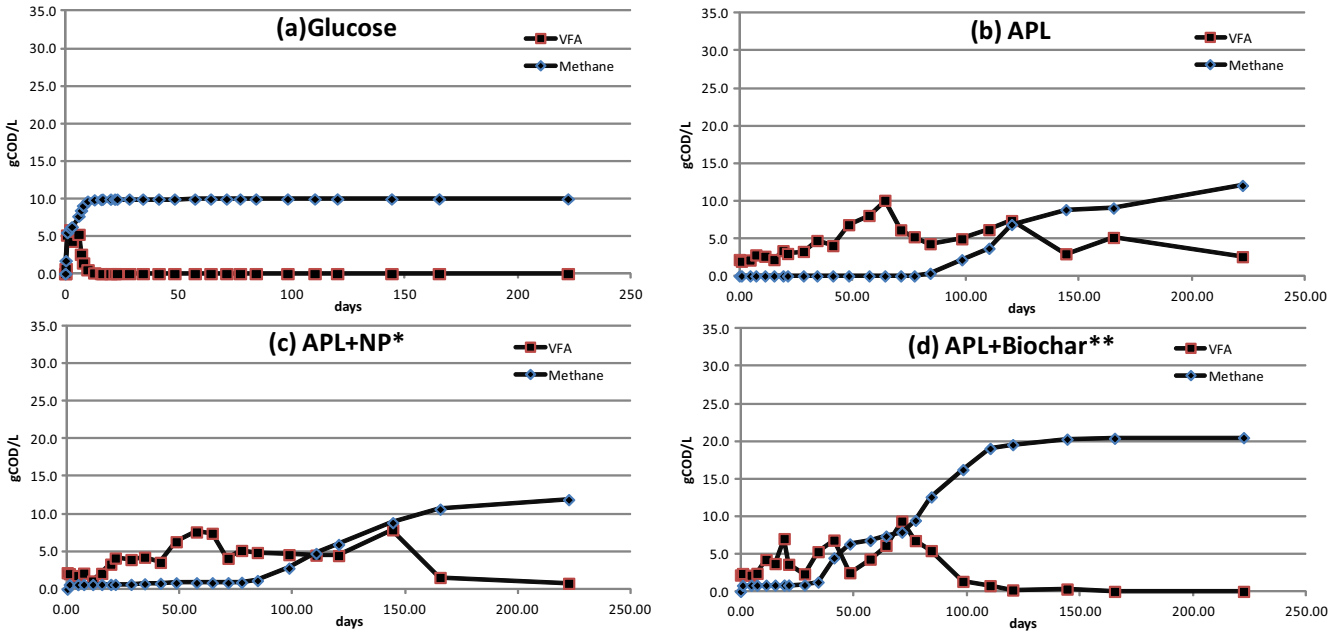

Fig. 3. Trend of methane and VFA generated during anerobic digestion of glucose ((a) 12 gCOD l^{-1}), APL (b), APL supplemented with nutrients ((c) 35 gCOD l^{-1} APL) and APL:biochar in 1:1 ratio ((d) 35 gCOD l^{-1} APL).

acidification immediately after the substrate addition, and final methane yield equal to 85% of theoretical one after less than 10 days.

For APL digestion, in all cases, acidogenesis and methanogenesis occurred clearly later than for glucose, and no significant methane production was found in the first 30 days. Without biochar or nutrients, even in absence of methane production, a marked increase in VFA content was observed during the first 60 days, with a peak value of about 10 gCOD l^{-1}, corresponding to 28% of the COD of the APL. After the peak, a slow decrease of VFA to 5–8 gCOD/L was observed just before the methane production, which occurred at 85th days from the beginning of the test and takes 140 days until exhaustion. The speed of methanogenesis was quite low, with a rate of about 0.07 $gCOD_{CH_4}$ l^{-1} d^{-1}. The overall methane production was 12 ± 2 $gCOD_{CH_4}$ l^{-1} which corresponds to a methane yield of 34 ± 6% (mean ± standard deviation, $n = 2$) compared with the theoretical yield. In general, as expected, the mode and extent of biogas production in test performed with sole APL was close to the average behavior observed in the 1 l tests.

In the test performed with APL and supplemented nutrients (nitrogen and phosphorous) the trend observed for VFA and biogas was roughly the same, with marked increase in VFA in absence of

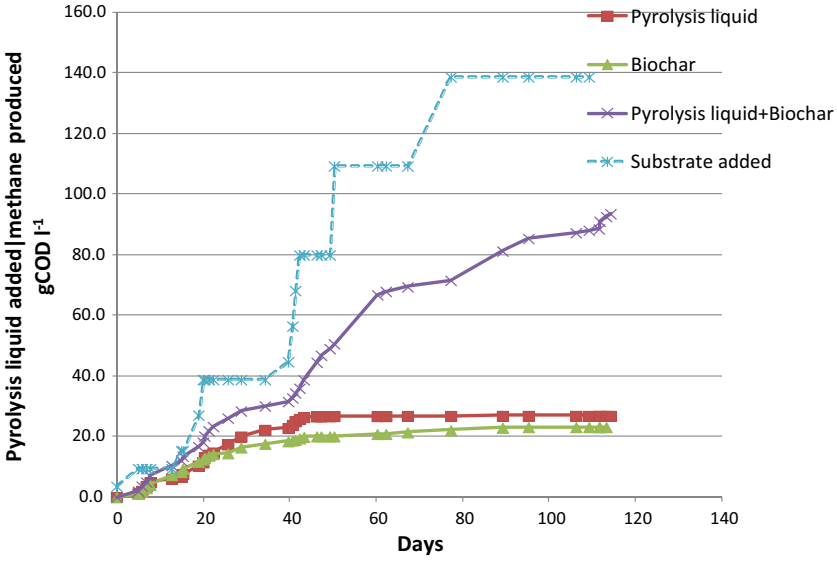

Fig. 4. Results from semicontinuous tests of anaerobic digestion of pyrolysis liquid in presence of biochar. All data are shown in gCOD l^{-1}, the comparison between substrate added (dashed line) and methane produced (solid lines) provide the yield for each test at a certain time.

any methane generation, followed by recovery at the 85 day and methane production. Stable methane production, and decrease of VFA to less than 1 gCOD l^{-1}, occurred in the subsequent 80 days, showing an average rate of methane production of 0.1 $gCOD_{CH_4}$ l^{-1} d^{-1}. Finally, the methane production was 10 ± 2 $gCOD_{CH_4}$ l^{-1} which corresponds to a methane yield of 29 ± 6% compared with the theoretical yield, and can be considered comparable to that obtained with the sole APL. Differently, in the test performed with APL and biochar, even if the production of VFA occured almost with the same rate as in the other tests, this was accompanied by earlier methane production at the 35th day. The gas production remained stable from 35th to 110th day with average rate of methane production 0.2 $gCOD_{CH_4}$ l^{-1} d^{-1} and apparent exhaustion of biological process (no VFA and no methane generation) at the 144th day. The overall methane production was 20 ± 5 $gCOD_{CH_4}$ l^{-1} which corresponds to a methane yield of 60 ± 15% of theoretical one. Therefore, yield was doubled when biochar were added. The reasons for the observed effect can be more than one, but anyway the presence of this material allows to sustain a meaningful methanogens activity after just 35 days and even in presence of 8 gCOD l^{-1} of VFA, whereas the nitrogen and phosforous supplementation had negligible effect. This findings suggests that the unbalance in anaerobic process observed in the previous paragraph is probably not only due to a simple macro-nutrient imbalance but to other, more complex, inhibition mechanism. In fact, it is interesting to notice that, in all the micro-scale tests, the concentration of VFA in APL fermentation rapidly reached values over the inhibition levels reported for both hydrolysis and methanogenic reaction (Niu et al., 2013; Siegert and Banks, 2005). It is known that hydrolysis and acidogenesis, which is less sensitive to VFA, is inhibited slightly later (IC_{50} = 8 g l^{-1}) than methanogenesis (IC_{50} = 6 g l^{-1}) and this can, in principle, explain the VFA unbalance and, consequently long lag phase observed in the test (Siegert and Banks, 2005). Therefore, an hypothesis can be that APL could act as a more stronger inhibitor of methanogens than for acidogens, and this could create the detected VFA accumulation, that ultimately caused the long lag phase and the consequential poor yield. From the obtained data, biochar actually eased this phenomena and this could ultimately speeded up the conversion. This could be done in different ways: (i) by decreasing the inhibition effect of APL and keeping the equilibrium VFA level slightly lower, (ii) by decreasing the inhibition of VFA towards VFA consuming microorganisms.

3.3. Semi-continuous digestion of APL in presence of biochar

Since the lag phase can be partially due to the unbalance due to adaptation to complex substrate, semi-continuous set up was tested for digestion of this substrate. Daily addition of APL was provided in order to overcome the unbalanced acidogenesis and inhibition observed in the batch tests and to adapt the system more gradually. Fig. 4 shows the obtained results. In comparison to batch tests performed in previous section, starting with small amount of APL (6 gCOD l^{-1} d^{-1}), after 5 days it was possible to obtain the first gas production. With low initial APL input both biochar loaded and biochar-free reactor showed a relatively short lag phase. After 20 days, the amount of APL added was increased with average daily additions of 15 gCOD l^{-1} d^{-1} (in two or three inputs per week). Following this increase in APL input, the reactor without biochar, gradually stopped the biogas production. Analogously, production of methane from biochar alone stopped at 20 $gCOD_{CH_4}$ l^{-1} after 40 days (corresponding to 0.03 $gCOD_{CH_4}$ $g^{-1}_{biochar}$) indicating that biochar degradation is not relevant under the conditions of this study.

Interestingly, the reactor fed with APL and biochar continued to produce bio-gas with a relatively stable methane content for all the duration of the experiment. Even after a total 6 g of APL in 30 ml (corresponding to 140 gCOD l^{-1}) added for the duration of the study (120 days) no signal of intoxication was revealed and a relatively stable methane production was observed. Considering the entire duration of the test, the final specific averaged methane production was about 0.2 ml_{CH_4} $ml^{-1}_{reactor}$ d^{-1}, with peak values of more than 1 ml_{CH_4} $ml^{-1}_{reactor}$ d^{-1} in the middle of the study and a final methane yield (once subtracted the "blank" containing only biochar) of 65 ± 5% with respect to theoretical one. Considering that the system is not yet optimized, these rates are encouraging and noticeably close to those expected for the digestion of easily digestible substrates. On the opposite, in the case of the system without biochar, in analogy to that observed in paragraph 3.1, but with stronger all-or-nothing evidence, production of gas never recovered for the duration of the study. Looking at the literature, it was found that chars could enhance the conversion of difficult product in the anaerobic digestion environment. In particular, it

is known that char-like materials (like activated carbons or charcoals) can decrease the toxicity of certain inhibitors: like phenols (Hanaki et al., 1997), coal gasification wastewaters (Fox et al., 1990), and ammonia (Mumme et al., 2014). Biochar can partially absorb inhibitors, buffer the pH and provide support for the microorganisms. The findings of this work seem to confirm this positive effect and confirm that biochar could be envisaged as a low cost "catalyst" for anaerobic processes, especially in presence of potential inhibitors.

4. Conclusions

This paper investigated the anaerobic digestion of aqueous fraction from intermediate pyrolysis, APL. As it is, this substrate is challenging and causes inhibition phenomena that slow down the conversion that end up with low methane yield. Nevertheless, if the biochar co-produced by pyrolysis is added in the system, and if the system is acclimatized gradually, APL can be converted with an acceptable rate. APL can be considered a model for the water soluble substances in fast pyrolysis oil and gasification wastewater. Therefore, this option could open up new interesting pathways for the integration of biological and thermochemical processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.09.021.

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